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Addressing Food Safety and Marketing Concerns Through Microbial
Reduction Strategies for Michigan Blueberries.

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Overview:

Microbial safety and quality of blueberries are critical concerns to all segments of the marketing chain. Although the blueberry industry has not been negatively impacted by any widespread outbreaks of illness, food safety concerns have indirectly affected producers and marketers in the form of buyer demands for microbial testing and increasingly stringent microbial specifications. Most Michigan blueberries are frozen for use in processed products such as pies, muffins, and jams. Buyers of frozen blueberries are now demanding microbial testing in order to assure that the product does not exceed their specifications for standard plate count, yeasts, molds and various bacterial pathogens. However, microbial specifications vary considerably from buyer to buyer. Blueberry producers have observed large variations in microbial levels that do not seem to relate consistently to either field conditions or handling procedures. The industry clearly needs a better understanding of the factors contributing to high microbial levels, as well as effective microbial reduction strategies.

Objectives:

The specific objectives of this project were to (1) assess levels of microbial contamination at various points during blueberry ripening in the field, harvesting, handling, cleaning and packaging and (2) compare the efficacy of sanitizing treatments.

This work was conducted by scientists in the departments of Food Science and Human Nutrition, Plant Pathology, and Horticulture, at Michigan State University. Funding partners were the U.S. Highbush Blueberry Council, which represents producers throughout the United States, MBG-Marketing (Grand Junction, MI), which is a grower-owned cooperative marketing over half of Michigan blueberries, and ICA TriNova (Forest Park, GA), which develops food sanitizing products.

Results:

Field Factors Affecting Microbial Levels: We sampled green fruit and ripe fruit (before the first and second harvest) from different field locations in western Michigan (12 in 2003 and 6 in 2004). Fruit washings were analyzed for the presence of yeasts, molds, and bacteria to determine which organisms were prevalent on blueberry fruit surfaces and if populations increased over time.

Results showed that fungi, yeasts, and bacteria populations increased considerably during the fruit development and harvest period. Populations varied widely between field sites. A clear reason for the differences was not found, although overhead irrigation may promote populations. Among the fungi, *Cladosporium acutatum*, the causal agent of anthracnose fruit rot of blueberries, was predominant, as it was found in nearly all fields, and occupied 95-100% of the fungal populations. This suggests that seasonal control of

fruit rot may help in reducing mold counts after harvest. *Cladosporium*, *Epicoccum*, *Alternaria*, and *Phoma* species were also detected on fruit surfaces. These fungi are common in the environment and live on dead and dying plant material. Some of the common yeasts on blueberry fruit surfaces were identified as *Aureobasidium pullulans*, *Sporobolomyces* sp., *Sporidiobolus* sp., *Cryptococcus* sp., *Bulleromyces* sp., *Filobasidium* sp., and *Rhizosphaera* sp. These yeasts are also common on plant surfaces and are generally not harmful to humans. Plant-associated bacteria were also common, e.g. *Bacillus subtilis*. The yeasts and bacteria on blueberry fruit surfaces may actually be beneficial as antagonists to or competitors with blueberry fruit rot pathogens.

Representative microbes were tested for the production of the extracellular enzymes amylase, pectinase, and cellulase. Amylase has been shown to reduce viscosity of baked goods when blueberries were added to batter that was refrigerated before baking. The other enzymes could potentially lead to breakdown of the fruit itself, but their relationship to fruit quality or quality of baked products is unknown. All 22 fungal isolates produced amylase while 11 produced cellulase; 16 isolates produced some form of pectinase with one isolate producing only polygalacturonase and 14 producing only pectin methylesterase. Of the 32 yeast isolates, 24 produce amylase and 1 produced pectin methylesterase. None of the yeast isolates produced cellulase. Of the 33 bacterial isolates, 22 produced amylase and 20 produced cellulase. None of the bacterial isolates produced pectinase. These findings show that most of common fruit-associated microbes are able to break down starch. The relationship between microbial levels and quality of baked goods needs to be studied to develop specifications based on sound scientific data.

Effect of Harvesting and Handling on Microbial Levels: Fruits were also sampled for two seasons from twelve processors in Michigan at five points during harvesting and processing operations: pre-harvest; pre-processing; post-blower, post-water tank; and before freezing. The numbers of mesophilic aerobic bacteria, coliforms, *Escherichia coli*, yeast and molds were determined using standard methods.

Results showed some consistent trends. Microbial populations generally increased by 1 log or more between pre-harvest and post-harvest. This may have been the result of contamination during mechanical harvesting in addition to the incubation time between harvest and the beginning of processing. Most blueberries were held for 12 hours or more before processing began. Microbial populations decreased less than one log as they exited a chlorinated water tank (~10 to 200 ppm chlorine). Blueberries generally contained higher microbial levels after processing than prior to harvest. Several findings can be helpful in reducing microbial levels. First, blueberries should be processed as soon as possible after harvest in order to minimize microbial growth. This is particularly true if harvested berries are from later pickings with higher microbial loads. Secondly, chlorination of water in the processing line does not effectively sanitize blueberries. This is primarily due to the short contact times that are typically less than 1 minute.

Samples of fresh blueberries were frozen within 8 h of collection and microbiologically examined following 3 and 6 months of storage at -20°C. Microbial populations decreased 0.5 to 0.7 logs after either 3 or 6 months of frozen storage.

In addition to the fruit, samples from the environment (swab and water samples) were also assessed for levels of microbial contamination. Before and after processing of the fruit, 18 swab samples (100 cm²) were collected from the conveyor belt surface entering the blower and the packaging area. In addition, water samples (50 ml) were collected from the water tanks before and after fruit processing. Microbial populations significantly increased on conveyor belts and in the water tank during fruit processing.

Sanitizing Treatments. Various microbial reduction strategies were evaluated including sodium hypochlorite (100 and 200 ppm), chlorine dioxide (3 and 5 ppm), proprietary organic acids and chlorine dioxide gas in addition to a water control. Fresh blueberries were inoculated by immersion in cocktails of bacteria, molds, and yeasts, dried for 24 h, and then exposed to the sanitizers for up to 5 min. Sodium hypochlorite and aqueous chlorine dioxide were marginally effective, providing reductions of 0.5 to 1.5 logs. An organic acid solution was most effective, reducing levels of bacteria, yeasts and molds 2.52, 3.77 and 3.72 log CFU/g at the highest concentration (0.9%). Although the efficacy of these sanitizers can be increased by lengthening the time berries are in the water tank (i.e., slowing down the processing line), this is problematic during the peak of the harvest season. Another promising sanitizing approach was gassing with ClO₂ after harvest and before processing. In preliminary work using buckets, we exposed inoculated blueberries to 0.16 mg ClO₂ gas/g fruit for 12 h. Results showed that populations of bacteria, yeasts and molds decreased more than 3 logs. Thereafter, 600 lb lots of naturally contaminated fruit exposed to 0.13 mg ClO₂ gas/g fruit for 12 hours. In these scaled up experiments, bacteria, yeasts and molds decreased 2.33, 1.63 and 0.48 logs, respectively. The gassing treatment (0.19 mg ClO₂ gas/g fruit for 12 h) did not affect berry appearance, aroma, texture, flavor or overall acceptability.

Current and Future Benefits from this Work.

This work demonstrated that microbial loads on blueberries could be reduced by timely harvesting to avoid over-ripe fruit and by prompt processing and freezing which will minimize microbial growth. Several sanitizers tested in water baths were only marginally effective in reducing populations, but treating 600 lb pallets of blueberries with chlorine dioxide gas showed considerable promise as a sanitizing treatment for processed and perhaps fresh fruit.

Future Research Needs

Additional chlorine dioxide gassing strategies need to be developed and further refined for sanitizing blueberries. The enzymatic capabilities of the various yeasts found on blueberries also need to be further understood since some of these yeasts can break down starch, pectin, and cellulose, thus affecting pie setting and quality. In particular, the relationship between microbial levels and baked product quality needs to be quantified to develop sound specifications for blueberry fruit.

Publications from this Work

Popa, I., E.J. Hanson, A. Schilder, E.C.D. Todd, and E.T. Ryser. 2005. Levels of microbial contaminants in highbush blueberries before, during, and after processing.

Abstract P3-09, International Association of Food Protection Annual Meeting, Baltimore, MD, Aug 14-17.

Popa, I., E.J. Hanson, A. Schilder, E.C.D. Todd, and E.T. Ryser. 2005. Inactivation of bacteria, yeasts, and molds on highbush blueberries using chlorine dioxide sachets. Abstract T1-07, International Association of Food Protection Annual Meeting, Baltimore, MD, Aug 14-17.

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